

**REMARKS**

**I. Introduction**

Applicants respectfully request reconsideration and withdrawal of the rejections set forth in the Office Action.

Claims 2, 3, 4, 7, 8, 12, 13, 18, 19, 23, and 42 have been canceled, without prejudice or disclaimer thereof. Applicants reserve the right to prosecute the subject matter of these claims in this or another application.

In addition, claims 1, 5, 6, 9-11, 14-17, 21, 22, and 24 have been amended, and claims 46-59 have been added to the application. Details of the amendments and new claims are provided in the following discussion.

Because the foregoing amendments do not introduce new matter, entry thereof by the Examiner is respectfully requested.

**II. Summary of the Claimed Invention**

The claimed invention provides retroviral vectors for efficient delivery of a nucleotide sequence of interest (NOI), or nucleotide sequences of interest (NOIs), to one or more target sites. The retroviral vectors of the claimed invention also provide a system for effective expression of the NOI or NOIs at one or more target sites. The claimed invention further provides retroviral vectors incorporating specific safety features that enhance the *in vivo* application of these vectors.

Specifically, the claimed invention provides retroviral vectors comprising splice donor (SD) and splice acceptor (SA) sites. The SD and SA sites are not functional when the retroviral vector of the claimed invention is in the form of a pro-vector, and are only rendered functional following reverse transcription of the pro-vector such that a retroviral vector is formed (see Application at page 39, lines 6-11).

For the avoidance of confusion and for clarity, presented below is a detailed account of the events leading to the conversion of non-functional SD and SA sites to functional SD and SA sites.

#### **A. The Retroviral RNA Genome of A Virus and Pro-Virus**

Upon entry into a susceptible host cell, the retroviral RNA genome is reverse transcribed to DNA by a virally encoded reverse transcriptase enzyme (RT) that is physically carried inside the virus particle. This DNA is transported to the host cell nucleus where it subsequently integrates into the host genome. At this stage, the genome is typically referred to as the pro-virus. Each retroviral genome encodes genes such as *gag*, *pol*, and *env* (see Application at page 8, lines 19-26). *Gag* encodes the internal structural proteins, *pol* encodes reverse transcriptase (RT) and other enzymes, and *env* encodes for outer envelope proteins (*env* can be substituted with a non-retroviral envelope protein coding gene such as VSV-G, MLV amphotropic *env*, feline leukemia *env*, HA of influenza virus, etc. See Application at page 48, lines 21-24; page 75, lines 5-7).

In the pro-virus, these genes are bracketed by specific sequences called long terminal repeats (LTRs). The LTRs play an important role in pro-viral integration into the host genome and also in transcriptional initiation. The site of transcription initiation is at the boundary between U3 and R in the left hand side LTR (see Application at page 8, lines 8-14; Figures 12, 13, and 14 identified by filled in arrows). Encapsidation of the retroviral RNAs occurs due to the presence of the packaging signal - *psi* ( $\psi$ ) located at the 5' end of the viral genome.

#### **B. Structure of the LTRs and Relationship to the Retroviral Genome**

The LTRs are identical sequences that can be divided into three elements, called U3, R, and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is derived from the sequence unique to the 5' end of the RNA. Thus, in its natural environment, the infectious retroviral

RNA genome can be represented as follows:

(5') R - U5 - *gag, pol, env* - U3 - R (3')

### **C. The Reverse Transcription Process**

Reverse transcription of the genomic RNA into double stranded DNA involves two jumps of the reverse transcriptase enzyme from the 5' terminus to the 3' terminus of the template molecule. It is well documented (thus not requiring detailed description) that the two jumps lead to a duplication of the R-U5 and U3-R sequences and the concomitant translocation/transfer of one copy of each of these sequences to the opposite ends of the genome. There is no duplication and/or translocation of sequences contained between the LTRs.

As a result, a copy of the U3-R region is translocated from the 3' end to the 5' end and a copy of the R-U5 region is translocated from the 5' end to the 3' end of the genome, while a copy of the template sequences is left behind. These sequences then occur fused in tandem, U3-R-U5, on both ends of the viral DNA, forming the long terminal repeats (LTRs). Thus the viral DNA genome can be represented as follows:

(5') U3 - R - U5 - *gag, pol, env* - U3 - R - U5 (3')

See Coffin et al., *Retroviruses*, pp. 122-123 (Cold Spring Harbor Laboratory Press, USA, 1997).

### **D. Effect of Placing a Splice Donor Site Between U3 and R and Downstream of a Splice Acceptor Site**

Thus, if a splice donor site (SD) is placed between U3 and R (*i.e.*, at the 3' end of the genome (*see* Application at page 68, line 8 and below) and downstream of a splice acceptor site (SA) site (located in the sequences between the LTRs; *see* Application at page 44, lines 22-24; page 68, lines 12-16; and Figures 12, 13, 14, 17, and 18), upon reverse transcription the SD site will be translocated/transferred to the 5' end of the pro-virus (*i.e.*, the 5' LTR region of the retroviral genome) and thus upstream of the SA (*see* Application at page 68,

lines 7-10). Schematically, the translocation of SD can be illustrated as follows (emphasis added):

(5') R - U5 ----- SA ----- U3 - SD - R (3') (pro-vector in primary target cells)

↓      Reverse Transcription/Transduction

(5') U3- SD -R-U5 ----- SA-----U3 - SD - R - U5 (3') (vector in secondary target cells)

The primary target cells (packaging cells) contain transcriptional units coding for *gag*, *pol*, and *env*, as well as a transcriptional unit which encodes for a retroviral pro-vector genome capable of being packaged into a retroviral particle (*see* Application at page 43, lines 23-30; and page 45, lines 5-8).

Splicing of the retroviral pro-vector genome would be prevented because the SD site is located downstream of the SA site (*see* the schematic above and Application at page 39, lines 6-10; and page 44, lines 5-9). The SD site is located at the 3' end of the retroviral genome upstream of the R-region, *i.e.*, within the 3' LTR region (*see* Application at page 44, lines 19- 21; page 68, lines 5-8; and Figures 2, 4, and 10), while the SA site is located between the two LTRs and is therefore not subject to translocation (*see* Application at page 44, lines 22-24; and page 68, lines 12-16).

The retroviral particles produced from these packaging cells will carry a functional RT enzyme and will contain pro-vector genomes which will upon internalization into a secondary target cell be reverse transcribed by the RT enzyme to form a retroviral vector which can integrate into the host cell genome (*see* Application at page 39, lines 10-11; page 44, lines 9-13; page 45, lines 8-10; and Figures 17 and 27c).

In addition, the reverse transcription will lead to the U3-SD-R cassette being 'inherited' to the 5' end and RNA transcripts expressed will contain an SD site located at the 5' terminus which is upstream of the SA site (*see* Application at page 44, lines 9-13). This translocation and rearrangement of the LTR sequences will render functional SD and SA in the retroviral vector (*see* Application at page 44, lines 24-28; and Figure 18).

### **III. The Office Action**

#### **A. Rejection of the Claims Under 35 U.S.C. § 112, First Paragraph – Written Description**

Claims 1-19, 21-24, 30, and 42 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors at the time the application was filed has possession of the claimed invention. In particular the Examiner alleged that “[t]he combination of elements in the vector of claim 1 cannot be found in the specification.” Office Action at page 3. The Examiner also alleged that the specification does not describe certain features of claim I, namely a splice donor site within the 5'LTR region and a splice donor site “at” the 3' U3-R region of the retroviral vector and, accordingly, these features add new subject matter. Office Action at page 3. Applicants respectfully traverse this ground for rejection.

In contrast to the Examiner’s assertion, Applicants’ specification describes the construction of MLV retroviral pro-vectors which contain non-functional splice donor and splice acceptor sites which, following reverse transcription, can be converted to functional splice donor and splice acceptor sites (*see* Application at Example 1, pages 71-72). Figure 2 (page 68, lines 5-10) details the construction of the 3' long terminal repeat-splice donor site (3'LTR-SD). Figure 3 (page 68, lines 12-17) describes the inclusion of the splice acceptor (SA) site and the sequence data of the vector which contains the non-functional splice donor (3' LTR-SD ) and splice acceptor sites, which are only created functional upon reverse transcription.

A similar strategy was used for the construction of the EIAV pro-vectors, where the conversion of the nonfunctional splice donor and splice acceptor sites to functional splice donor and splice acceptor sites is described in detail in Example 2 (page 75, lines 12-30). The legend of Figure 6 (paragraph bridging pages 68-69) describes the first step of the cloning strategy such that the insertion of a splice donor site at the CMV/R junction of the EIAV LTR generates the CMV-SD-R-U5 construct. The next step in the cloning strategy is the addition of a splice acceptor site, which is described in Figures 7 and 8. The final stage of

the cloning strategy is joining the vector carrying the splice donor site with the vector carrying the splice acceptor site, which is described in Figure 9. Consequently, similar to the MLV pro-vectors, the splice donor and splice acceptor sites in the EIAV pro-vector are not functional in the primary target site. This is due to the fact that the pro-viral genome that is transfected into the primary target site carries an SD site which is located in the 3'LTR, and as such is downstream of the SA site. The retroviral particles produced from the primary target site will carry an RT enzyme and an RNA genome which will upon reverse transcription be converted to DNA and a concomitant translocation of the U3 -SD -R sequences to the 5'LTR and thus upstream of the SA (see schematics above).

In both examples (MLV and EIAV), although only experimentally demonstrated for EIAV, the splice donor and splice acceptor sites will only be rendered functional following reverse transcription (see below and Application at Example 2, lines 5-7; and Figures 14 and 17).

Therefore, if a splice donor site is positioned at the 3' end of the genome, *i.e.*, at the junction between U3 and R of the pro-vector, the SD site is downstream of the splice acceptor site and consequently prevents splicing. However, after transduction (reverse transcription), the 3' end of the pro-vector, *i.e.*, the U3/SD/R region, is duplicated and translocated to the 5'LTR of the retroviral vector (see Figures 14, 17, 18, and 27c). Thus, expression of the first NOI is achieved only upon reverse transcription when the correct topographic re-positioning of the SD and SA sites is achieved, which in turn facilitates correct splicing and gene expression.

The Examiner has acknowledged that the present application describes an example where the selective expression of the hygromycin gene pair, or the hygromycin-p450 gene, is expressed in producer (primary target site) or transduced cells (secondary target site), respectively (Example 2, lines 5-7; and Figure 14). Other examples are also provided. For instance, Example 6 describes the construction of a conditional expression vector for P450 in which the gene coding for the P450 protein is arranged in two halves such that only after transduction is the correct splicing achieved, which in turn allows P450 gene expression (see Figure 18).

Thus, Applicants were in possession of the retroviral vectors as claimed and, therefore, the Examiner is kindly requested to retract this ground for rejection of the claims.

**B. Rejection of the Claims Under 35 U.S.C.  
§ 112, First Paragraph - Enablement**

Claims 1-19, 21-24, 30, and 42 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly not providing an enabling disclosure for any nucleotide sequence of interest (NOI). The Examiner also rejected Applicants' arguments that one of skill in the art would be able to compensate for cryptic splice sites by interfering with and altering the sites as taught by the art, and alleged that at the time of filing it was unpredictable whether a gene contained cryptic splice sites. Office Action at page 4. Applicants respectfully traverse this ground for rejection.

**1. At the Time the Claimed Invention was Made, One  
of Ordinary Skill in the Art Would be Able to  
Compensate for Cryptic Splice Sites**

As noted in Applicants' prior response, means for identifying and eliminating cryptic splice donor/acceptor sites, such as sequence analysis, were well known in the art prior to Applicants' claimed invention. *See e.g.*, Sebillon et al., *NAR*, 1995, 23:3419025 and Maruyama et al., *Eur. J. Biochem.*, 1995, 232:700-05, provided with Applicants' prior response.

Moreover, it was also well known prior to Applicants' claimed invention that removal of cryptic splice sites can improve or optimize performance. Examples described in publications were given in Applicants' prior response. For example, one reference reported that removal of a cryptic splice site significantly improved GFP performance. Another described that the modification of a plasmid sequence to eliminate a cryptic splice event optimized the plasmid's performance.

Accordingly, because those skilled in the art at the time of filing could readily detect and manage cryptic splice/donor sites, the application is enabling for more than just the working examples. Applicants therefore respectfully request withdrawal of this rejection.

## **2. Alleged Discrepancy Between Claim 1 and Claims 4 and 13**

The Examiner alleged that there was a discrepancy between claim 1 and claims 4 and 13 in that claims 4 and 13 require expression of the NOI, while the language of claim 1 does not require expression of a protein. This issue has been addressed by amending claim 1 and deleting claims 4 and 13.

## **3. Rejection of Claim 5 for Alleged Lack of Enablement Regarding a Vector Capable of Providing a Therapeutic or Diagnostic Effect**

Claim 5 was rejected as the specification allegedly lacks enablement for a vector capable of providing a therapeutic or diagnostic effect. Office Action at page 5. Specifically, the Examiner alleged that the specification does not disclose examples substantiating the claim that NOIs delivered by the retroviral vectors of the present invention have a therapeutic or a diagnostic effect. Applicants respectfully disagree with the Examiner's analysis and conclusion.

The claimed invention provides retroviral vectors comprising functional SD and SA sites such that the vectors can be used as vehicles for delivering NOIs to a target site. The vectors of the claimed invention have diverse applications that are dependent on the functions the NOIs are specifically designed to perform, such as for example, chemically selectable markers, color markers, and other therapeutic and/or diagnostic functions (*see* Application paragraph bridging pages 32 and 33; and page 35). It is well documented that administering a suitable pro-drug along with an appropriate pro-drug activating enzyme can be used for treating a subject (*see also* Application at page 35, lines 20-30). Therefore to be enabled, Applicants would need to demonstrate that the vectors of the claimed invention can indeed deliver and be able to efficiently express in a target site NOIs which encode a prodrug activating enzyme.

Applicants have fulfilled this requirement by showing that the vectors of the claimed invention can deliver and efficiently express a pro-drug converting enzyme in a target site (*see* Application at page 35; Example 2, page 77, lines 5-7; and Figure 14). The Examiner has indeed acknowledged the example described in Figure 14, thus recognizing that the vectors of the claimed invention can deliver and differentially express a selectable marker product in the primary target site and a therapeutic gene in the secondary target site. Therefore, a skilled worker taking into account the art and the teachings of the present application by reference to its description would be able to use the vectors of the claimed invention in therapy and diagnostics. The Examiner is therefore respectfully requested to withdraw this ground for rejection.

**C. Rejection of the Claims under 35 U.S.C. § 112, Second Paragraph**

Claims 1-19, 21-24, 30, and 42 were rejected under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Office Action at page 6. Applicants respectfully traverse this ground for rejection.

**1. Applicants' Amended Claim 1 is Definite**

Claim 1 was rejected as it allegedly remains indefinite "how the 'retroviral vector comprising . . .' relates to the 'retroviral pro-vector' or how the retroviral vector is made." Office Action at page 6.

In addressing the issue of the correlation between the "retroviral vector comprising . . ." and the "retroviral pro-vector . . .", Applicants note that the retroviral pro-vector exists before RT activity and the retroviral vector is obtained after RT activity. The interrelationships between the retroviral vectors and the retroviral pro-vectors of the claimed invention are extensively described above in Section II. In addition, the language of Applicants' amended claims distinguishes between the retroviral vectors and the retroviral pro-vectors of the present invention.

Numerous terms and phrases of claim 1 were rejected as being unclear. Applicants' courteously submit that claim 1, as amended, is definite and addresses the Examiner's concerns.

**2. Clarity of Applicants' Claims Regarding the Phrases  
"A First Nucleotide Sequence . . ." and "A Second  
Nucleotide Sequence"**

The Examiner stated that the wording of the phrase "a first nucleotide sequence ("NS") containing a functional splice donor site" and "a second nucleotide sequence ("NS") containing a functional splice acceptor site" can adequately be described as "a functional splice donor" and "a functional splice acceptor site".

In traversing this ground for rejection, Applicants note that the term "nucleotide sequence" encompasses more than just splice donor or splice acceptor sites. For example, the NS may comprise an immunological molecule or an immunoglobulin (*see* Application at page 25, lines 28-30; and 26, lines 1-6).

It is respectfully submitted that Applicants' claims, as amended, address the Examiner's concerns.

**3. Clarity of the SA and SD Site in the Pro-Vector and Vector**

The issue of clarity of the SA and SD sites in the pro-vector and the vector has been addressed in the amended set of claims. For example, claim 1 has been amended to recite "a functional splice donor site" and "a functional splice acceptor site," as suggested by the Examiner. Claim 46 recites the retroviral vector of claim 1 further comprising first and second nucleotide sequences, encoding the functional splice donor site and the functional splice acceptor site, respectively. Accordingly, Applicants' claim language referring to SA and SD sites is definite.

**4. Rejection of Claims 1 and 2 for Reciting the Term "Containing"**

Claims 1 and 2 were rejected as being unclear for reciting the term "containing," as the Examiner believes that it is unclear whether this term defines open or closed language.

As amended claim 1 does not recite the term "containing," and claim 2 has been cancelled, this ground for rejection is moot.

**5. Rejection of the Claims for Recitation of the Phrase "Non-Functional Donor Splice Site"**

The Examiner objected to the phrase "non-functional donor splice site" in claim 2. As claim 2 has been cancelled, this ground for rejection is moot.

**6. Rejection of Claim 4 for Failing to Limit the Invention of Claim 3**

Claim 4 has been cancelled, without prejudice or disclaimer thereof. Accordingly, this ground for rejection is moot.

**7. Rejection of Claim 5 on the Grounds that a Nucleic Acid Does not Provide a Therapeutic or Diagnostic Effect**

The Examiner rejected claim 5 as "a nucleic acid does not provide a therapeutic or diagnostic effect – a protein provides" such effect. Office Action at page 9.

Contrary to the Examiner's assertion, it is incorrect to suggest that only proteins can provide a therapeutic or diagnostic effect. There are numerous examples where nucleic acid molecules, such as anti-sense RNA and transdominant negative mutants, have been used in therapy. In addition, oligo or polynucleotide sequences can be used as probes for hybridization analysis in diagnosis.

Thus, Applicants' second NOI can carry a sequence which codes for a protein, but just as importantly, it can carry a nucleotide sequence, both of which can be used in therapy or diagnostics (*see* Application page 35, lines 1-11).

Moreover, a given NOI can code for more than one element. For example, the first NOI can code for one or a plurality of products which are capable of conferring selectability, such as for example, resistance to antibiotics, different fluorescent or luminescent tags, etc. The first NOI can *also* carry one or more elements which are essential for retroviral particle production, such as the retroviral genome packaging signal *psi* ( $\psi$ ) (*see* Application at the

paragraph bridging pages 32-33; and page 33, lines 23-25). Therefore, from the context of the description, it is clear that the term "comprises" as used herein refers to a sequence which carries information for one or more elements. Thus, a NOI can comprise multiple sequences, not all of which must be expressed to confer selectability or help in the retroviral packaging process. As such, claim 5 as amended is definite.

#### **8. Rejection of Claim 6**

The Examiner objected to claim 6 because "a nucleic acid does not provide selectability." Office Action at page 9. Applicants respectfully disagree.

Although selection can be achieved though the expression of proteins, there are certain markers which can be attached/hybridized directly to nucleotide sequences and used as selectable markers. *See* Wilhelmsson et al., *Antisense Nucleic Acid Drug Dev.*, 11(4):265-70 (2001); and Nielseu PE, *Curr. Opin. Biotechnol.*, 10(1):71-5 (Feb. 1999). Thus, contrary to the Examiner's assertion, a nucleic acid can provide selectability.

In addition, claim 6 was rejected for recitation of the phrases "a part thereof," "essential elements," and "is or comprises." Office Action at page 9. As these phrases have been deleted from the claim, these grounds for rejection are moot.

#### **9. Rejection of Claim 8 for Reciting the Phrase "A Part Thereof"**

Claim 8 was rejected as being allegedly indefinite for reciting the phrase "a part thereof." Office Action at page 9. While Applicants respectfully disagree, claim 8 has been amended to delete this phrase. Accordingly, this ground for rejection is moot.

#### **10. Rejection of Claims 9 and 10 for Recitation of the Phrase "the First NS"**

The Examiner alleged that it was unclear in claims 9 and 10 if the phrase "the first NS" refers to the first NS of the retroviral vector or the first NS of the pro-vector. Office Action at page 10. Claim 9 has been amended to state that the functional splice donor site is from a virus, and claim 10 has been amended to state that the functional splice donor site is from an intron. As amended claims 9 and 10 are definite, this ground for rejection is moot.

### **11. Rejection of Claim 14**

Claim 14 was rejected as being allegedly unclear for recitation of the phrases “the second NS,” “one or more additional NOIs,” and “such that additional NOIs may be inserted.” Office Action at page 11. Claim 14 has been amended to recite a retroviral vector in which “the functional splice acceptor site is upstream of a multiple cloning site such that one or more additional NOIs may be inserted.” It is respectfully submitted that this claim language is definite and, therefore, this ground for rejection is moot.

### **12. Rejection of Claims 15-17**

Claims 15-17 were rejected as being allegedly indefinite because “it is unclear how the second NS can comprise both a splice acceptor site and encode an immunoglobulin.” Office Action at page 11.

Claim 15 has been amended to recite a retroviral vector in which the “functional splice acceptor site is from a nucleotide sequence coding for an immunological molecule;” claim 16 has been amended to recite the retroviral vector of claim 15 in which the immunological molecule is an immunoglobulin; and claim 17 has been amended to recite the retroviral vector of claim 16 in which the immunoglobulin is from an immunoglobulin heavy chain variable region. It is respectfully submitted that this claim language is definite and, therefore, this ground for rejection is moot.

### **13. Rejection of Claim 21**

Claim 21 was rejected for the recitation of the phrase “wherein the vector or pro-vector is a murine oncoretrovirus or a lentivirus retroviral vector or pro-vector.” Office Action at page 12. Claim 21 has been amended to recite “a retroviral vector” in which “the vector is a murine oncoretrovirus vector or a lentivirus retroviral vector.” It is respectfully submitted that this claim language is definite and, therefore, this ground for rejection is moot.

### **14. Rejection of Claim 22**

Claim 22 was rejected as being allegedly indefinite because “it is unclear [whether] ‘the vector refers to only the ‘vector’ in claim 21 or both the vector and pro-vector.” Office

Action at page 12. In view of the amendments to claim 21, it is clear that the phrase "the vector" in claim 22 refers to a vector and not a pro-vector. This ground for rejection is moot.

**15. Rejections of Claims 7, 12, 13, 18, 19, 23, and 42**

Claims 7, 12, 13, 18, 19, 23, and 42 were rejected for various reasons. As these claims have been cancelled, these grounds for rejection are moot.

**IV. Conclusion**

The present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

If there are any fees due in connection with the filing of this Amendment, please charge the fees to our Deposit Account No. 19-0741. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Date: Aug 23, 2002

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**Marked Up Version of Claims with Markings to Show Changes**

1. (Three Times Amended) A retroviral vector comprising a 3' and 5' long terminal repeat (LTR), the retroviral vector further comprising:
  - (a) [a first nucleotide sequence ("NS") containing] a functional splice donor site; [and]
  - (b) [a second NS containing] a functional splice acceptor site;
  - (c) a first nucleotide sequence of interest ("NOI") flanked upstream by the functional splice donor site and downstream by the functional splice acceptor site; and
  - (d) a second NOI downstream of the functional splice acceptor site;  
wherein the functional splice donor site is within the 5' LTR of the retroviral vector  
[wherein:
    - (i) the functional splice donor site and the functional splice acceptor site flank a first nucleotide sequence of interest ("NOI");
    - (ii) the functional splice donor site is upstream of the functional splice acceptor site; and
    - (iii) the retroviral vector is formed as a result of reverse transcription of a retroviral pro-vector, wherein the retroviral pro-vector comprises:
      - (a) a first nucleotide sequence ("NS") containing the splice donor site; and
      - (b) a second NS containing the splice acceptor site; wherein the first NS is downstream of the second NS; such that the retroviral vector comprising a first NS containing a functional splice donor site and a second NS containing a functional splice acceptor site is formed as a result of reverse transcription of the retroviral pro-vector].
5. (Four Times Amended) [A] The retroviral vector according to claim 1 [3] wherein the second NOI, or the expression product thereof, is [or comprises] capable of providing a therapeutic agent or a diagnostic agent.

6. (Four Times Amended) [A] The retroviral vector according to claim 1 wherein the first NOI, or the expression product thereof, [is or] comprises a selectable marker[any one or more of an agent conferring selectability], a viral [essential] element, [or a part thereof,] or a combination [combinations] thereof.

9. (Four Times Amended) [A] The retroviral vector according to claim 1 wherein the functional splice donor site [first NS] is from a virus [viral NS].

10. (Three Times Amended) [A] The retroviral vector according to claim [9] 1 wherein the functional splice donor site [first NS] is from an intron [or a part thereof].

11. (Three Times Amended) [A] The retroviral vector according to claim 10 wherein the intron is the small t-intron of SV40 virus.

14. (Four Times Amended) [A] The retroviral vector according to claim 1 wherein the [second NS] functional splice acceptor site is [placed] upstream of a multiple cloning site such that one or more additional NOIs may be inserted.

15. (Four Times Amended) [A] The retroviral vector according to claim 1 wherein the [second NS] functional splice acceptor site is from a nucleotide sequence coding for an immunological molecule [or a part thereof].

16. (Three Times Amended) [A] The retroviral vector according to claim 15 wherein the immunological molecule is an immunoglobulin.

17. (Three Times Amended) [A] The retroviral vector according to claim 16 wherein the [second NS is a nucleotide sequence coding for] immunoglobulin is from an immunoglobulin heavy chain variable region.

21. (Four Times Amended) [A] The retroviral vector according to claim 1 wherein the vector [or pro-vector] is a murine oncoretrovirus vector or a lentivirus retroviral vector [or pro-vector].

22. (Three Times Amended) [A] The retroviral vector according to claim 21  
wherein the vector is a MMLV, MSV, MMTV, HIV[-l], or EIAV retroviral vector.

24. (Four Times Amended) [A] The retroviral particle obtained from a retroviral  
vector according to claim 1 wherein the retroviral particle comprises a retroviral vector  
comprising a functional splice donor site within its 5' long terminal repeat (LTR).